

# The NIH CATALYST

A PUBLICATION FOR NIH INTRAMURAL SCIENTISTS

NATIONAL INSTITUTES OF HEALTH ■ OFFICE OF THE DIRECTOR ■ VOLUME 9, ISSUE 2 ■ MARCH-APRIL 2001

## Angiogenesis Research AIMING TO CONTROL A DOUBLE-EDGED SWORD

by Fran Pollner

A few years ago, angiogenesis was hot news—albeit some times manufactured and distorted—as cancer patients responded to a front-page story in the lay media that implied cancer could be cured by angiostatin and endostatin, two angiogenesis inhibitors.

The news was manufactured in that angiogenesis wasn't "news"; it was a biological phenomenon whose process and implications for human health had been studied for decades, perhaps most notably in the laboratory of Judah Folkman at Children's Hospital in Boston. It was distorted because, caveats notwithstanding, the context and quotes surrounding the reported mouse findings invited instant extrapolation to human primary and metastatic cancer.



The Angiogenesis Foundation

Clarifications ensued and eventually the noise subsided. Angiogenesis research, however, did not subside. At NIH, intramural research aimed at understanding angiogenesis, and how to inhibit or promote it in different disease settings, has been ongoing—and was the subject of a plenary session at the last NIH Research Festival.

New blood vessel growth, or its absence, can have both desirable and damaging results. William Stetler-Stevenson, chief of NCI's Extracellular Matrix Pathology Section, observed in his introductory overview of this "rapidly expanding, controversial field." Angiogenic sprouting

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## Hot Methods: Protein Profiling BEYOND GENOMICS TO CLINICAL PROTEOMICS

by Lance Liotta, MD, PhD, NCI  
Emanuel Petricoin III, PhD, FDA

Although DNA is the information archive of the cell, proteins do all the work. They operate through a complex network of interactions and post-translational modifications that cannot accurately be predicted by gene transcript profiling.

The proteome is not a static entity. It is different in each cell type and changes from one minute to the next, depending on the cellular microenvironment and the physiologic state of the cell. Thus, the proteomic challenge is much more than just cataloging all the proteins encoded by the expressed genes.

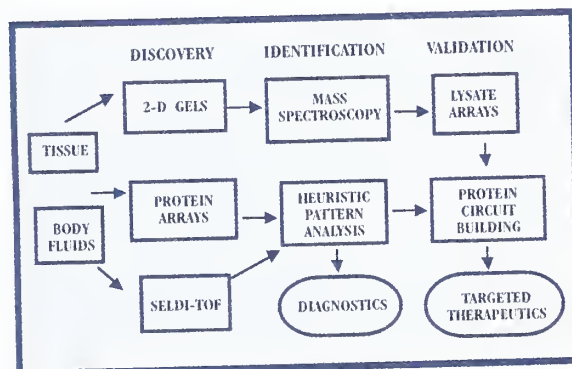
The true goal of proteomics—even grander than sequencing the genome—is nothing less than producing a complete wiring diagram of the protein networks of a cell, in health and under the influence of disease<sup>1</sup>.

### Tissue Proteomics Project

Three years ago, we established a joint research initiative between the NCI and the FDA called the Tissue Proteomics Project. The goal of this initiative has been to originate and complete technology for studying proteomic networks and signal pathways in small quantities of microdissected human tissue cells directly from biopsy specimens.

In contrast to the expanding list of biotech companies and consortia moving into proteomics, our goal has been immediate patient-based clinical applications. We have originated a series of technologies to extract and analyze the pattern of proteins and determine the activation state of known signal pathways using microdissected human tissue or small samples of patient serum.

Our analytical tools are divided into two classes: *protein microarrays* that are



NCI-CBER/FDA Tissue Proteomics Initiative

used to profile the pattern of known proteins, and *high-throughput biochips* that can rapidly read out protein patterns, even if the identity of the proteins is unknown. We are using these tools to apply proteomics to samples from clinical trials and epidemiologic screening.

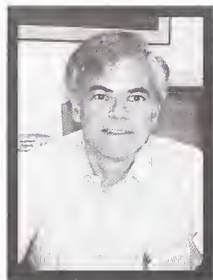
We believe advances in proteomics applied to tissue and body fluids will herald a new era in clinical research. Clinical investigators will monitor whole proteomic patterns of information, not just the concentration of one marker. Analysis of the patterns obtained before and after pharmaceutical treatment, or over the course of disease progression, could lead to insights about how an ex-

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## UPFRONT AND PERSONAL: RECRUITING FELLOWS TO NIH



Michael Gottesman

**A**t last count, NIH had more than 3,000 postdoctoral and research fellows on our rolls—a cohort of individuals who play a large and vital role in conducting research at NIH. There is no question that the quality of NIH research reflects the quality of our postdoctoral fellows. How can we ensure that the very best fellows come to work at NIH?

There are two sides to the answer to that question. First, we have to use wise selection criteria and good judgment when we offer positions. Second—and this is where most of this column will focus—we have to look as attractive to prospective postdocs as they look to us. Often, that means simply ensuring that they in fact *know* what sort of place NIH is.

Fellows choose to come to NIH primarily because they are familiar with, and impressed by, the work of our scientists. We make contact with fellows every time we publish a paper, give a lecture at a university, or speak at a meeting. Speaking engagements, especially, offer the opportunity not only to display one's own scientific wares but also to extol the research environment at NIH.

That said, trainees still tend to stay in academia for postdoctoral experiences because they are networked into universities through their professors, their mentors, and their own comfort level. One way to raise the comfort level of potential postdocs who might not think of a government institution as an appealing research setting is to have them visit NIH—and see for themselves that this is a real campus with a decidedly academic bent. We know that students who have been here are much more likely to come back as fellows. Moreover, some of our efforts to recruit students from disadvantaged backgrounds involve visits to the NIH campus, and these always pay off with increased recruitment of high-quality fellows.

The research environment is one component of a decision to come to NIH; our reputation for caring about the career development of our trainees is another. Our recent emphasis on improved training and mentoring of our fellows can only help us recruit postdocs. We have anecdotal information suggesting that fellows trained at NIH do extremely well in the academic and industrial job markets or, if they are visiting fellows, return to their home countries and become scientific leaders. We will eventually be able to access data about the jobs NIH trainees take when they leave here, but in the meantime, it is definitely a useful strategy to let people know what happens to fellows who have left your lab.

There are several institutional strategies that can be used to improve fellows' quality of life. More

and more, fellows have families and other responsibilities and, given the increasing length of postdoctoral training (based both on job conditions and the growing complexity of biomedical research), they deserve higher stipends and better living conditions. The various NIH intramural campuses offer attractive environments in which to live, and our stipends will be increasing 6 percent this year—with similar increases anticipated in subsequent years until a competitive stipend is reached. We hope to be competitive with postdoctoral positions in other disciplines and reduce the suffering traditionally associated with the postdoc training period.

NIH is also sponsoring more daycare slots for infants and children: A new daycare center is going up on Wisconsin Avenue near the Natcher building, and another is planned on the Old Georgetown Road side of the campus off Center Drive. There is also a large daycare center at Executive Boulevard that has a shorter waiting list. We may never satisfy all the childcare needs of the NIH community, but at least the waiting list can get short enough that slots don't have to be reserved before conception.

Almost all of our intramural programs now have dedicated training personnel who can help in the recruitment of potential postdocs, and NCI has developed an office devoted to this endeavor, including helping new fellows find housing and making them feel welcome. The increasing spirit of collaboration and intellectual stimulation on the NIH campus, reflected in our lecture series, activities of special interest groups, and state-of-the-art resources (not to mention the gradually improving quality of our research space), has to have a positive influence on recruitment of fellows.

As to the other issue—our ability to make a reasoned judgment before offering someone a position—one complaint that I hear frequently is that we have no way to evaluate potential visiting fellows before they come here, especially from developing countries where we do not have longstanding ties to the scientific establishment. We have been exploring various options to aid in this evaluation, but the most useful is to accelerate the development of networks with senior scientists working in these countries. As the scientists we have trained return to their home countries, opportunities to establish these networks grow. In addition, I strongly recommend that potential fellows be interviewed whenever possible, preferably in person in their home country or by bringing them to NIH for a visit, before committing a postdoc position at NIH.

I welcome your ideas on how best to enhance recruitment of fellows to NIH.

—Michael Gottesman

Deputy Director for Intramural Research

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## CATALYTIC REACTIONS

### On the NIH Health Disparities Center

I think that one general area of study for the new Health Disparities Center should be the interactions between genetics and environment. For example, are African-Americans, Hispanic-Americans, Native Americans, Asian-Americans, and European-Americans on the same high-fat diet equally likely to become obese? If not, are there predictive differences in genes affecting things such as steroid metabolism?

—Roland Owens, NIDDK

## Peer Review Program

The Center for Scientific Review (CSR) is establishing the Review Internship Program to provide scientists with training and experience in some aspects of scientific research administration. This program is initially limited to NIH intramural scientists interested in gaining first-hand experience with the peer review process; it may be extended in the future to other scientists. Applications are now being accepted for positions that will start August 1, 2001; the **application deadline is April 20**. Program coordinators are Rona Hirschberg and Anita Miller Sostek.

A forum on the new Review Internship Program will be held **April 2 from 1:00-3:30 pm** in Building 31, Room 6C-7. General inquiries can be directed to Mary Elizabeth Mason at 301-435-1114.

TTY-TDD users may call Lori Stoller-Cruz, CSR, at (301) 301 594-7891.

Additional information about the program, application forms, and other requirements can be found on the CSR intranet web site:

<http://csrevnet.csr.nih.gov/internship/internship.htm>.

Alternate formats are available on request. ■

## Mind-Body Meeting

The NIMH Integrative Neural Immune Program is hosting a conference at the Masur Auditorium in Building 10 on the "Science of Mind-Body Interactions: An Exploration of Integrative Mechanisms," **March 26-28** (9:00 a.m. to 4:30 p.m. the first two days; 9:00 a.m. to 12:20 p.m. the last day).

Other sponsors are NINDS, the OIR, and the John D. and Catherine T. MacArthur Foundation. Eleven other NIH components are co-sponsors.

Conference agenda, speakers, objectives, and other information are available at the conference website:

<http://www.mindbody.org>.

There is no fee for NIH campus scientists, but advance registration is required—either by fax: 312-641-8555 or by e-mail:

[register@mindbody.org](mailto:register@mindbody.org).

or online at the website:

<http://www.mindbody.org>.

## Pharmacogenetics

The inaugural meeting of the newly established Pharmacogenetics Research Network and Knowledge Base will be held **April 25, 2001**, in the Lister Hill Auditorium.

Sponsored by NIGMS and several other NIH components, the meeting will feature research presentations as well as panel discussions on both ethical issues and industry relations. There is no charge to attend.

For a printable meeting flyer and a list of speakers, topics, and registrants, visit the NIGMS Pharmacogenetics Research Network homepage:

<http://www.nigms.nih.gov/pharmacogenetics.html>,

where online registration is available and encouraged. For further details, contact the meeting organizer, Rochelle Long, at 301-594-1826, or e-mail:

[rochelle\\_long@nih.gov](mailto:rochelle_long@nih.gov).

## Astrobiology Dialogue

The NASA Astrobiology Institute (NAI) and the National Institutes of Health are holding a day-long joint symposium **April 2, 9:00 a.m. to 5:30 p.m.**, at the Clinical Center's Masur Auditorium. The purpose of the symposium is to initiate an NAI-NIH dialogue at the scientist-to-scientist level that is expected to lead to new collaborative research on cosmic aspects of life (see *The NIH Catalyst*, May-June 2000, "Astrobiology and the Search for Origins," page 1).

This first symposium will focus on

—Extremophiles

—Biofilms

—Oxidative damage and biological forms of iron

Future interactions are certain touch on such areas as remote sensing to the role of water in life.

For more information and a complete agenda and to register online (not mandatory but requested), visit the website:

<http://nai.arc.nasa.gov/JointSymposium>.

## Careers Seminars

The NIH Fellows Committee, Office of Research on Women's Health, and Office of Education are sponsoring a series of seminars on "Scientific Careers in the New Millennium." They are held at Lipsett Amphitheater in Building 10 from 9:30 a.m. to noon.

The next two in line are on:

■ **"Writing," Thursday, March 29**  
With Nancy Touchette (freelance) and Crispin Taylor (*Science NextWave*)

■ **"Policy," Thursday, April 12**  
With Christine Grady (deputy director, NIH Bioethics Department) and Deborah Stine (COSEPUP)

A reception in the Visitor's Center follows each event.

Reservations are not required, but seating is limited. For more information, contact:

[aaains@box-a.nih.gov](mailto:aaains@box-a.nih.gov).

## SCIENTIFIC MISCONDUCT: HOW IT IS HANDLED IN THE INTRAMURAL RESEARCH PROGRAM

by Joan P. Schwartz

**T**he scientific community and the community at large rightly expect adherence to exemplary standards of intellectual honesty in the formulation, conduct, and reporting of scientific research.

Allegations of scientific misconduct are taken seriously by the NIH. The process of investigating allegations must be balanced by equal concern for protecting the integrity of research as well as the careers and reputations of researchers. The NIH Committee on Scientific Conduct and Ethics (CSCE) has spent most of a year adapting for use in the intramural program the model guidelines provided by the Office of Research Integrity (ORI) of the Department of Health and Human Services on how to handle allegations of scientific misconduct. Several key concepts underlie the guidelines:

■ **Timeliness.** A prompt response to an allegation serves two purposes: If the allegation proves true, it helps to minimize any harm to the public that could result; if it proves false, the names of those incorrectly accused will have been cleared as expeditiously as possible.

■ **Confidentiality.** Allegations of misconduct that prove to be untrue, even if they were made in good faith, can damage careers and have a chilling effect on research. Confidentiality helps protect innocent people who were incorrectly or unjustly accused, as well as those who brought the allegations.

■ **Fairness.** Fairness affords all those who become involved in scientific misconduct cases the opportunity to participate in the process; it seeks to protect innocent participants from adverse consequences.

### Defining Circumstances

In this article, I will briefly describe how allegations of scientific misconduct are handled in the NIH IRP—but first some background is in order on the reasons the White House Office of Science and Technology Policy (OSTP) saw fit to develop a new definition of scientific and research misconduct that would be uniform for all federal agencies.

Several very high-profile cases of scientific misconduct were in the news over the past decade or so, generating considerable interest in the scientific community and the public at large—not to mention the halls of Congress, where the appropriators of funds for such agen-

cies as NIH and the National Science Foundation were not pleased that taxpayer money might be supporting research marred by fabrication, falsification, or plagiarism.

One result of all this interest was the realization that each federal agency had a somewhat different definition of what constituted scientific misconduct. Such discrepancies could make for quite confusing proceedings should allegations of misconduct be leveled at, say, a university faculty member with grants from both NIH and NSF.

The project under suspicion would have to be picked apart to determine which aspects were funded by which agency's grant and which definition of misconduct would need to be met by which allegations.

Furthermore, the former Public Health Service definition included the vague catchall phrase "or other practices that seriously deviate from those that are commonly accepted within the scientific community for proposing, conducting, or reporting research."

The new policy, issued by the OSTP—  
<[http://www.ostp.gov/html/001207\\_3.html](http://www.ostp.gov/html/001207_3.html)>

—establishes a uniform definition for the federal government. Scientific/research misconduct is

"... fabrication, falsification, or plagiarism in proposing, performing, or reviewing research, or in reporting research results.

**Fabrication** is inventing data or results.

**Falsification** is manipulating research materials, equipment, or processes, or changing or omitting data or results such that the research is not accurately represented in the research record.

**Plagiarism** is the appropriation of ideas, processes, results, or words of another person without giving appropriate credit, including ideas, processes, results, or words obtained through confidential review of research proposals and manuscripts.

Research misconduct does not include honest error or honest difference of opinion."

Along with the loss of the "other practices" clause, the other big change is that peer review is now clearly included in the definition.

### Research Ethics Case Discussions

As described in a column in the last issue of *The NIH Catalyst* (January-February 2001, "Protecting the Integrity of the Scientific Enterprise," page 2), each of you will have the opportunity to participate in some type of case discussion this year that will present real instances of activities that can occur in a laboratory or other research setting.

Part of the discussion will center around which of those activities constitute misconduct and why, thereby affording you the opportunity to understand these issues at a practical level. Some of you may have observed activities or behaviors in your own work environment that you felt were questionable but didn't know how to handle. These case discussions should help in such real-life situations.

The CSCE prepared a short, easily understood version of the official Guidelines for handling misconduct. It is currently available on the web at

<<http://www1.od.nih.gov/oir/sourcebook/ResEthicsCases/sm-booklet.htm>>

and will be provided to each of you in written form when you have your case discussion.

### Allegation and Response

The process for handling allegations of scientific misconduct (see flow chart) begins with someone's suspicion that he or she has evidence of or has observed research misconduct. Such a concern may be shared confidentially with a trusted person, such as a lab chief, scientific director, or other NIH official.

Formal complaints alleging research misconduct must be made in writing and contain sufficient details to make clear the nature of the activity, including a description of the facts, events, and circumstances that led to the allegation. The signed document is sent to the Agency Intramural Research Integrity Official (AIRIO), who carries out allegation assessment.

Currently, I am the AIRIO, and anyone who feels uncomfortable approaching their lab chief or scientific director can come directly to me and be assured of confidentiality. I can be reached at Building 1, Room 135; phone: 496-1248; e-mail: <[jps@helix.nih.gov](mailto:jps@helix.nih.gov)>.

The AIRIO decides whether the allegation warrants an Inquiry, which is

preliminary fact-finding to determine whether there is enough evidence behind an allegation or apparent instance of scientific misconduct to warrant moving to the next level of response—an Investigation. An Inquiry Committee, consisting of at least three scientists with expertise in the area, will interview those involved and decide whether to recommend an Investigation. If the allegation describes events or conduct that may pose a threat to human or animal research subjects, a violation of safety regulations, financial irregularities, discrimination, sexual harassment, or criminal activity, the AIRIO will notify the appropriate NIH official.

The Investigation is the formal examination and evaluation of all relevant facts to determine if scientific misconduct has occurred, and, if so, to determine the person(s) who committed it and the seriousness of the misconduct. The committee that carries this out consists of at least five scientists, one of whom is a peer of the person(s) against whom the allegation was raised (the respondent).

proof (a preponderance of the evidence); whether the misconduct was committed intentionally, knowingly, or recklessly; and whether it represents significant departure from accepted practices of the relevant research community. If the committee decides that misconduct has occurred, it will also recommend NIH sanctions.

The NIH ARILO (Agency Research Integrity Liaison Officer, currently Ron Geller, OD) will decide whether to accept the report, make a finding of misconduct, and impose the recommended NIH sanctions. Possible NIH sanctions include removal from a particular project; placing a letter of reprimand in the individual's NIH personnel file; special



Fran Pollner

*Joan Schwartz, OIR assistant director and a NINDS section chief, is also the NIH AIRIO (Agency Intramural Research Integrity Official), to whom formal allegations of scientific misconduct are brought and who carries out the initial assessment of the merits of the allegation.*

or termination of employment.

The final step in the process is a review by ORI, which then makes recommendations to the assistant secretary for health (ASH) on possible PHS sanctions. It is the ASH who would make the final decision as to whether misconduct has occurred and, if so, impose the PHS sanctions (such as debarment from serving on NIH study sections or receiving NIH grants).

The scientist has the right to appeal this decision to the Departmental Appeals Board.

### Some Stats

This brief summary is intended to provide the key elements involved in handling allegations of scientific misconduct. How big a problem is scientific misconduct at NIH?

In the six years I have been involved, we have had 15 allegations of scientific misconduct that proceeded to an Inquiry or Investigation—and there was a finding of misconduct in two of these cases.

I would be surprised if any of you have heard about even one of them, unless you were directly involved. We believe that we have successfully incorporated the three key concepts—confidentiality, timeliness, and fairness—in our handling of these cases, but would be happy to hear suggestions for how to improve the process.

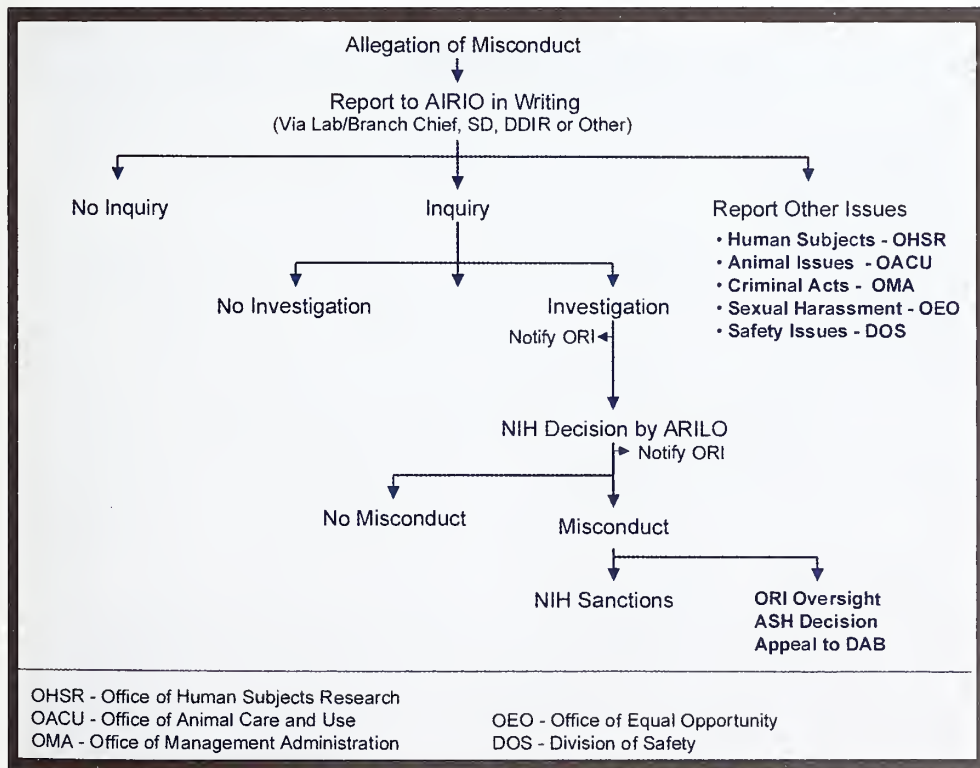
If you have questions, you can contact your IC representative on the CSCE. A list of CSCE members can be found at

<<http://www1.od.nih.gov/oir/sourcebook/comm-adv/sci-conduct.htm>>.

### Psst

In case you hadn't noticed, the archives of the online *Catalyst* are now just about complete. Except for the first year's issues (1993) and the first two of 1994, they're all there at

<<http://catalyst.cit.nih.gov/catalyst/>>.



The Investigation Committee must complete its work and prepare a report within 120 calendar days. The report will stipulate whether there is sufficient evidence for NIH to meet the burden of

monitoring of work; decrease in laboratory support (such as loss of a fellow or technical support position); probation; suspension without pay; denial of a raise in salary or a salary or rank reduction;

## RETROVIROLOGY AND THE RESERVOIRS OF HIV RESEARCH

by Annette Oestreicher

*NIH had a noticeable presence at the 8th Conference on Retroviruses and Opportunistic Infections, held February 4-8, 2001, in Chicago. Following is a summary of a few of the 70 or so reports from intramural investigators.*

The concept of HIV viral reservoirs has become increasingly important in the search for optimal antiretroviral therapy, as investigators attempt to stop or reduce therapy in aviremic patients. This subject formed the basis of a full symposium, which began with a comprehensive overview by Anthony Fauci, NIAID director and chief of the Laboratory of Immunoregulation.

Fauci recapped the independent findings of his own and two other teams that despite successful suppression of plasma virus with highly active antiretroviral therapy (HAART)—in many patients for years—replication-competent virus was still isolated from the resting CD4+ T cells of every patient tested. Rebound viremia, he said, invariably follows therapy discontinuation.

The “sobering realization of the recalcitrant nature of this reservoir,” the corollary that currently available therapies will have to be taken lifelong, and the surfacing of troublesome short- and long-term effects of HAART mandate a search for maneuvers to diminish the burden of the HAART regimen, as well as reduce the reservoir, Fauci said.

Several clinical centers have been experimenting with the strategy of interrupting therapy to lessen total drug exposure (and cost), as well as to increase drug adherence. Some experiments have demonstrated that such structured treatment interruption can enhance immunologic responses, leading to control of viremia in acutely infected persons. The situation is different, however, in those chronically infected, who likely have sustained substantial damage to their immune system.

Several interruption protocols—usually with treatment resumption when viral load increases to predetermined levels—have been tried with these patients. The aim, Fauci said, is to “elevate the immunologic setpoint to decrease acute viremia over time—[but] that is not happening.”

The NIAID team has taken a somewhat different approach to therapy interruption, evaluating the effect of struc-

tured intermittent therapy (SIT) at predetermined times, either two months on and one month off (long cycle) or seven days on and seven days off (short cycle). Fauci described the results of these studies, which were also presented at separate poster presentations.

### The Long and Short of SIT

The results of the trial of long-cycle SIT were reported by NIAID's Mark Dybul and his NIAID and industry colleagues.

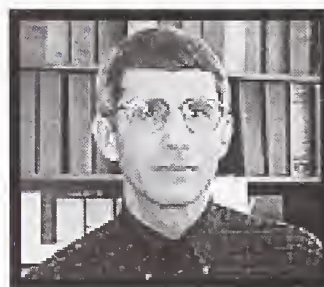
In this randomized, controlled study, 24 patients were placed on the intermittent regimen, and 23 were maintained on continuous treatment. All enrolled patients had plasma viral loads below 500 copies/mL for at least three months and below 50 copies/mL at screening; CD4+ T cells were greater than 300 cells/mm<sup>3</sup>.

Long-cycle SIT resulted in rebound HIV RNA in all 15 patients who'd completed between three and six cycles of eight weeks on and four weeks off therapy; viral load returned to screening levels in many by the end of the next eight-weeks-on cycle—or was no different from viral levels seen in the continuous treatment arm.

In general, then, rebound was followed by resuppression, there was no effect on CD4+ or CD8+ T-cell counts, and individuals on long-cycle SIT were able to reduce their drug exposure by one-third.

However, one of the 15 patients had evidence of genotypic and phenotypic decreased sensitivity to HAART during the third cycle. “That's a concern,” Fauci commented. Although the regimen has not been associated with any risks after one year, repeated increases and decreases in viral concentrations on long-cycle SIT could potentially have negative effects on the immune system, diminish susceptibility to HAART, and increase the risk of transmission during viremia, he suggested.

The same NIAID team reported more encouraging results with short-cycle—seven days on and seven days off—SIT. Twelve patients (with viral load and T-cell counts similar to those of the patients in the long-cycle SIT trial) were enrolled to receive short-cycle HAART for 24 months or until “failure,” defined



Fran Pollner

Anthony Fauci

as plasma HIV RNA above 500 copies/mL or a CD4+ T-cell count decline to 25 percent below baseline.

Not one instance of rebound viremia had occurred—up to 44 weeks at the time of the conference—in the 10 pro-

tocol-compliant patients, the team reported. Viral load remained below detectable levels. Both noncompliant patients experienced rebound.

There was no increase in proviral DNA or in the frequency of replication-competent HIV in CD4+ T cells; there was no accumulation of HIV RNA in the follicular dendritic cell network of lymph nodes; and there was no evidence of genotypic or phenotypic resistance in these patients.

However, the preliminary data also indicated that there was no evidence of selective amplification of HIV-specific immunity (with either long- or short-cycle SIT) or that the latent HIV reservoir was affected. Although the suppressive efficacy of short-cycle SIT, as well as the absence of untoward side effects and the 50 percent reduction in drug exposure, appears promising, Fauci noted, there is a great need for expanded clinical trials of this strategy.

### The IL-2 Boost

The addition of subcutaneous interleukin-2 (IL-2) to antiretroviral therapy (ART) to boost the immune system by expanding the pool of CD4+ T lymphocytes has been the subject of continuing NIH clinical studies conducted by NIAID clinical director Cliff Lane and Joe Kovacs, head of the AIDS section of the Clinical Center's Critical Care Medical Department, and their teams (see “IL-2 Immune Boost in HIV-Infected Patients . . .” *The NIH Catalyst*, September-October 1997, page 1).

Team members presented updates on these ongoing studies (as did other investigators worldwide, who are also testing this strategy). The long-term feasibility of this approach was evaluated in a longitudinal study of patients who had entered three separate prospective trials of IL-2 plus ART between 1993 and 1997. Findings from 63 of these patients, reported by Doreen Chait, Richard T. Davey, Jr., and their colleagues at the CC and NIAID, suggested

that substantially elevated CD4<sup>+</sup> T-cell counts can be sustained over long periods with a low frequency of intermittent IL-2 cycling and no adverse effects on viral load.

Attempts to blunt the associated inflammatory effects of IL-2 with the addition of prednisone to the regimen, however, reported by NIAID's Jorge Tavel and colleagues, resulted in a blunting of desirable immunologic effects as well.

In his overview, Fauci noted that IL-2, like the SIT strategy, does not appear to selectively enhance HIV-specific immunity.

### Acute HAART and the Long Haul

Another study out of Fauci's lab, reported by Tae-Wook Chun and colleagues, suggested that early initiation of HAART at HIV infection onset primes CD8<sup>+</sup> T cells to take on the task of long-term suppression of viral replication in latent viral reservoirs, independent of

cytotoxic T-lymphocyte (CTL) activity. The finding has implications particularly relevant in the context of long-term structured therapy interruptions.

Although CD8<sup>+</sup> T cells are known to participate in antiviral activity against HIV, their role in controlling HIV replication in the latent CD4<sup>+</sup> T-cell reservoir has been unclear, the investigators noted. They set out to evaluate the ability of autologous CD8<sup>+</sup> T cells to suppress viral replication in the resting CD4<sup>+</sup> T-cell reservoir.

Coculture experiments assessed cells from nine antiretroviral drug-naïve patients—six chronically infected patients and three long-term nonprogressors (LTNPs)—and eight patients with viral load below detection associated with HAART therapy.

Results showed that HIV was suppressed in the latent CD4<sup>+</sup> cell reservoir during coculture with autologous CD8<sup>+</sup> cells of LTNPs and with cells from

those receiving HAART, but not with cells from some of the chronically infected patients not receiving therapy. The mechanism of viral suppression was independent of CTL. RANTES, MIP-1 $\alpha$ , and MIP-1 $\beta$ —HIV-suppressive chemokines produced by CD8<sup>+</sup> cells—were found to play a major role in viral suppression in some LTNPs and in patients receiving HAART, but not in untreated chronically infected patients. However, it was an unidentified non-CC-chemokine factor secreted by the CD8<sup>+</sup> cells that exhibited the most potent antiviral activity and was found predominantly in patients in whom HAART was initiated shortly after the acute infection.

The presence of an unidentified soluble factor in patients in whom HAART was initiated during the acute phase of disease suggests that early initiation of therapy may play a role in containing viral replication during therapy interruption. ■

## Gut Level Responses

Leading off a symposium on HIV vaccine development, I. Jay Berzofsky, chief of the Molecular Immunogenetics and Vaccine Research Section of the NCI Metabolism Branch, reported new data from recently completed studies in macaques that demonstrated the importance of mucosal immunization in increasing the level of cytotoxic T lymphocytes (CTL) and decreasing viral load below detection in the gut.

Because most natural transmission of HIV occurs via mucosal surfaces, and because the lymphoid tissue in the colon and jejunum are important reservoirs of HIV, any hope of eradicating HIV from the body would require the presence of CTLs in the gut, Berzofsky emphasized.

Using selected SIV-HIV constructs, Berzofsky's team designed a study involving three groups of macaques: two of the cohorts received a peptide HIV vaccine, one subcutaneously and the other intrarectally, and the third was an adjuvant-only control group. After two immunizations, the groups were challenged intrarectally with HIV (SHIV-ku). Although all macaques became infected, there was a significant difference in the set point of viral load between those immunized intrarectally and those immunized subcutaneously. The intrarectal route was associated with a high level of CTLs in the colon and jejunum and better preservation of CD4<sup>+</sup> and CD8<sup>+</sup> counts. Berzofsky postulated that the presence of large numbers of CTL in the gut might better clear virus from the plasma. Immunization led to memory CTL in the colon and jejunum, which were then boosted during the viral challenge, an event that did not occur in the subcutaneously immunized macaques. Moreover, viral load was below detectable levels in the colon and jejunum of the intrarectally immunized macaques; the other two cohorts had a high viral load at those sites.

Berzofsky and his team, spearheaded by Igor Belyakov in Berzofsky's lab, in collaboration with NCI's Genoveffa Franchini and NIAID's Warren Strober and Brian Kelsall, undertook the macaque studies after they had demonstrated in mice—for the first time—that mucosal CTL are required to protect against challenge and that intrarectal, not subcutaneous, immunization is the required route. That work was done in collaboration also with NIAID's Bernard Moss, chief of the Laboratory of Viral Diseases.<sup>1-3</sup>

In another report, NIDCR's Sharon Wahl and MaryAnn Redford and NICHD's Patricia Reichelderfer—in collaboration with investigators at three medical centers—demonstrated that mucosal sites can harbor detectable levels of HIV RNA in women on antiretroviral therapy who have no detectable levels in peripheral blood. This study may have been the first to measure viral load in the oral cavity and genital tract as well as peripheral blood of women.

—A.O.

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CLINICAL PROTEOMICS  
continued from page 1

perimental drug works (or doesn't work) for an individual patient's disease and may reveal protein patterns that correlate with early disease or occult toxicity.

This article is part one of a two-part description of the proteomic tools under development in the NCI-FDA project. Here we will discuss "SELDI," a so-called protein biochip that can be used to rapidly generate protein molecular weight patterns—even though the protein identities are unknown. (Part two will discuss the use of protein microarray chips to monitor how drugs work in vivo.)

**Part One: SELDI**

Surface-enhanced laser desorption/ionization time-of-flight mass spectrometry (SELDI-TOF) is a promising technique for rapid protein-pattern analysis of serum and microdissected tissue specimens. A large amount of protein-pattern information is generated from a small sample in a short period of time, and no labeling (for example, radioisotope or fluorescent) of the proteins is required.

SELDI starts with stainless steel or aluminum-based supports, or "chips," 1–2 mm in diameter. These are precoated with hydrophobic (reverse-phase), normal phase, metal affinity, and cationic or anionic baits. Solubilized tissue or body fluids in volumes as small as 0.5–1  $\mu$ L are directly applied to these surfaces.

After a wash step, proteins and peptides of selected affinity are retained on the chip and analyzed by mass spectrometry—time-of-flight technology similar to MALDI (matrix-assisted laser desorption and ionization). Ionized proteins and peptides are recorded as they strike the detector plate and, depending on their size, travel down the vacuum tube in a time-dependent fashion (larger molecules take longer).

Proteins are recorded as mass signature "peaks" and displayed as a standard chromatograph. Routine identification of the protein peaks seen with this approach is not yet possible; that may change in the future, however, as the SELDI is coupled to instruments better adapted to protein microsequencing. Using SELDI, protein profiles can easily be obtained in minutes from as few as 25 to 250 cells. Furthermore, SELDI provides a complementary approach to 2D chromatography because SELDI is able to profile proteins regardless of their intrinsic hydrophobicity and has its best sensitivity (in the attomole range) to proteins below 15 kDa—a problematic size range for 2D-PAGE resolution.

Coupled to laser-capture microdissection (LCM)<sup>2</sup>, SELDI protein profiling is an important tool for the molecular finger-

printing of cancer cells from human tissue, shedding light especially on changes in protein expression in early premalignant lesions.

Protein expression profiling is rendered as a traditional mass chromatograph or as a density graph "barcode" (see figure, page 9). We obtained reproducible patterns of protein expression from microdissected patient-matched cells that consistently changed over the course of the malignant process in esophageal, prostate, colon, ovary, and breast epithelium procured by LCM.

Intriguingly, these patterns showed evidence of cancer-type specificity.

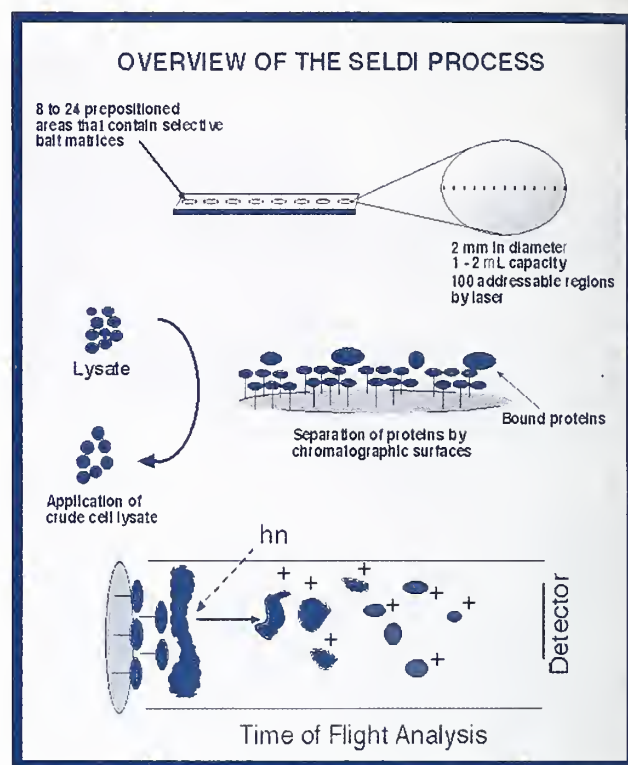
**Materials and Methods****■ Tissue Preparation and Staining.**

Frozen section slides, 8  $\mu$ m thick, are prepared as previously described<sup>3</sup>, except the proteinase inhibitor kit AEBSP (Boehringer Mannheim, Indianapolis) is added to the staining baths at a final concentration of 2 mM to inhibit proteases. With careful review of the histologic sections by a pathologist, each microdissection should have a greater than 95 percent purity.

**■ Laser Capture Microdissection.**

Stained tissue sections are subjected to LCM (Pixcell 100, Arcturus Engineering, Mountain View, Calif.; see *The NIH Catalyst*, November–December 1997, "Hot Methods"). Within five minutes of capture, microdissected cells are lysed directly with 10  $\mu$ L of an extraction buffer containing 1% weight-to-volume (w/v) Triton-X-100 (Sigma, St. Louis), 1% (w/v) MEGA 10 (ICN, Aurora, Ohio), 1% (w/v) octyl- $\beta$ -glucopyranoside (ESA, Chelmsford, Mass.), and 0.1% SDS (BIO-RAD, Hercules, Calif.) in a standard 1X phosphate-buffered saline (PBS).

**■ SELDI Analysis.** SELDI analysis is performed using an aliphatic reverse phase chip (H4 Protein Chip™, Ciphergen, Palo Alto, Calif.). The bait surfaces on the chip are pretreated with 1  $\mu$ L of acetonitrile (Sigma). Shortly before the acetonitrile completely evaporates, 1  $\mu$ L of the lysate is applied to the bait surface. The analyte is allowed to concentrate by air-drying followed by washing two times for 5 minutes in 1 x PBS. Next, 0.3  $\mu$ L of a saturated solution of 3,5-dimethoxy-4-hydroxycinnamic acid (sinapinic acid, Sigma, St. Louis, MO), the energy-absorbing molecule of choice, is applied to the



Liotta and Petricoin

*Analysis of biological mixture by ablation, ionization, and time of flight*

washed surface of the chip and allowed to crystallize.

**■ Reproducibility and Sensitivity of Protein Biomarker Profiling from Defined Cells.** To assess the reproducibility of the molecular weight signatures generated by the SELDI protein fingerprint of LCM-derived cells, 1,500 cells of esophageal normal epithelium were microdissected, lysed, and applied to a predefined bait surface on a hydrophobic interaction C18 biochip (Ciphergen), as outlined above. A data set was generated using the cumulative detection of proteins from 12 of the possible 100 different addressable regions within a single biochip surface region.

Each of these experiments was performed in triplicate (three separate cumulative groupings of 12 different areas of the same spot), giving a total of 36 data points for each protein peak analyzed.

Different peaks and shoulders were chosen for their diversity in relative intensity to one another. These were analyzed using normalization to a protein that appeared consistently in all experiments.

The reproducibility of the detection of the tissue proteins was quantitatively analyzed by comparing the relative proportionality of a subset of these peaks with one another.

Analysis of the reproducibility of the protein profile obtained from several independent applications of equivalent loadings of the same lysate was performed using a lysate of microdissected esophageal normal epithelium from the same pertinent sample.

In this study, the lysate from 30,000 cells from the same patient was applied to 20 different C18 biochips with hydrophobic interaction bait surfaces (for an average protein load of 1,500 cell equivalents per bait surface) and analyzed in triplicate (12 regions per data set), for more than 720 data points per protein analyzed.

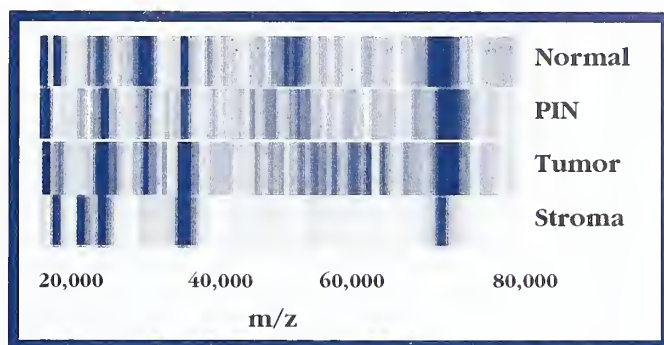
The coefficients of variance (less than 10 percent) and standard deviations generated from these analyses reveal that the molecular mass fingerprint of a given lysate can be reproducibly attained from any independent application.

We tested the sensitivity of protein detection by titrating lysates of microdissected normal esophageal epithelial cells from 1,500 to 0 cells/application. Each analysis was performed in triplicate.

The results of this type of experiment can be represented as a "virtual gel"—a gel-like display that takes the data from the mass chromatogram and presents them as if they were from a standard 1D-SDS-PAGE gel "stained" for proteins, with the molecular-weight ranges displayed at the same scale as that seen in the chromatogram (see figure above).

The lower limits of a reproducible protein fingerprint are in the range of 25 to 250 cells. When sensitivity is calculated as a product of cell-equivalencies, the detection limits become even more dramatic. This calculation is based on the fact that each individual bait surface on the protein biochip contains approximately 8,000 theoretically addressable regions, based on the area of a circle. Each of the individual protein profiles generated represents the cumulative detection of 12 different regions within one sample spot, so that the sensitivity of detection in terms of cellular equivalents is 12/8000th, or 0.15 percent, of the total lysate of the cells applied to the surface with-in one spot. This means that the biomarker protein profile in each reading represents the lysate of only two cell-equivalents!

Having achieved high sensitivity and reproducibility, SELDI was applied to microdissected prostate tissue—normal, premalignant, and invasive cancer from the same patient. It should be noted that these are microscopic lesions never before analyzed for their proteins. As shown in the figure, specific protein differences were uncovered, which were characteristic for each specific stage of cancer development, setting the stage for a new concept of molecular fingerprinting.



### Pharmacoproteomics of the Future

In conclusion, protein biochips can be used to generate protein fingerprints from microscopic cell populations directly from human tissue. This technology has attained a high degree of reproducibility, sensitivity, and specificity.

Tissue protein profiling may well become an essential component of pharmacoproteomics (patient-tailored therapies), improving therapeutic assessment, drug or surgical intervention strategies, toxicity monitoring, and disease diagnosis. ■

NOTE: Mention of a specific product in *The NIH Catalyst* does not imply endorsement. Failure to mention other products does not imply any opinion, positive or negative, about the products.

### Alert!

"The Next Step: Exploring the Proteome," May 21, daylong, Masur Auditorium. Speakers from Large Scale Biology Corporation, Celera, University of Michigan Center for Proteome Studies, University of Geneva, Institute for Systems Biology, Carnegie Mellon University, Matrix Science, Proteome, Inc., and NCBI. Sponsor: Mass Spectrometry IG and many institutes. Info: Sanford Markey 496-4022.

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### Instant Proteomics?

Just when you were getting the hang of potato chips, computer chips, and cDNA chips, along comes proteomics, and, naturally, the ProteinChip™—arrays of chromatographic spots to help you analyze proteins, in much the same way oligonucleotide microarrays allow you to look at gene expression.

So far, about six NIH labs have forked over the \$145,000 to acquire the Ciphergen ProteinChip™ reader, says Ciphergen rep Gerard Hoehn, who spoke at NIDCR on March 8. Depending on how busy a lab keeps the reader, it may also burn its way through \$30,000 to \$70,000 worth of chips in a year. Hoehn estimates that, worldwide, about 60 scientific papers have now been published about work that used the Ciphergen chips.

Hoehn said the chips could be used to accomplish a mouthwatering menu of protein-analysis tasks. The most crude would be separating a mishmash of 500 to 750 proteins from any complex biological sample on a 12 x 8 array. With samples of a mere 2,000 cells, researchers could thus go on a reasonably fast-paced fishing expedition for biomarkers—proteins whose characteristics (concentration, molecular weight, or binding properties) are altered in response to some event—a disease, toxin exposure, or age.

Closely related to a biomarker hunt would be protein expression profiling (see "Beyond Genomics to Clinical Proteomics," page 1)—using the chips to compare differences in protein expression in differ-

ent cell types. Analysis of signal transduction is a hot basic science area for protein chips, Hoehn said, including examination of critical posttranslational modifications of signaling molecules and identification of ligands for orphan receptors. They can also be used to detect and, with some extra fiddling, locate various "ations" on a protein—phosphorylation, glycosylation, ubiquitination—any posttranslational modification that alters molecular weight.

Before the chips can do their magic, they must first be "derivatized"—have their surfaces coated with an array of chemicals or covalently bound biological molecules, or baits, that will differentially trap proteins in a sample. Inorganic toppings come preloaded onto chip arrays (again, see main article). Biological toppings must be selected and attached by the investigator to a carbonyl diimidazole- or epoxy-coated chip. Possible additions could include antibodies, receptors, ligands, other proteins, or even DNA.

NIMH's Brian Martin, collaborating with FDA's Li-Shan Hsieh, has been test-driving the ProteinChip™ Platform—a machine on intermittent loan from the company. He finds it reliable, exciting, fun, and a pain, all at once. "There's no shortage of data. The problem is designing experiments so you can interpret the data and have [them] mean something," Martin says. He and Hsieh are looking for allergens in latex. Martin's also going after some putative transcription factors and receptors for scorpion toxins.

—Celia Hooper

## ANGIOGENESIS

continued from page 1

can compromise normal function, as in diabetic retinopathy, or compensate for compromised function, as in collateral vessel formation in response to coronary atherosclerosis. Insufficient vascularization is a concomitant of Alzheimer's and other dementias, increased vascularization a hallmark of tumor growth and metastasis.

Clinical trials at NIH involving angiogenesis have focused on angiogenesis inhibition in a variety of cancer settings and, Stetler-Stevenson summed up, are suggesting that this approach delays and diminishes—but does not eliminate—cancer progression.

### Testing the Limits

Vasostatin inhibited both angiogenesis and tumor growth in studies undertaken by Giovanna Tosato, head of the Molecular and Cell Biology Section at the NCI Department of Experimental Transplantation and Immunology—and the scientist who discovered vasostatin's activity and identified it as a fragment of calreticulin, a ubiquitous, intracellular protein that serves as a calcium depot.

Mice injected with Burkitt cells "displayed tumor restraint" in the presence of vasostatin, she reported, noting that vasostatin "did not abolish growth, but did delay it." Similarly, in studies with human-derived cells from melanoma, rhabdomyosarcoma, Hodgkin's lymphoma, Wilm's tumor, and colon and breast cancers, subcutaneous vasostatin injection inhibited endothelial cell proliferation, angiogenesis, and tumor growth.

Cytostatic rather than cytotoxic action of antiangiogenic agents against tumor cells was reported by other investigators as well, and there was general consensus among the speakers that angiogenesis inhibitors would not be effective as solo therapy but could theoretically work very well in combination with conventional cancer chemotherapeutic regimens.

The operating hypothesis in the lab of William Figg, chief of the Clinical Pharmacokinetics Section of the NCI Department of Developmental Therapeutics, is that "tumor cells release growth factors to recruit endothelial cells." Figg is an investigator in several NCI clinical trials of antiangiogenic agents; in the lab, he and his team have been dissecting the antiangiogenic properties of thalidomide and, he said, "We think we've identified

the active metabolite" of thalidomide that actually exerts the inhibitory effect on angiogenesis seen in clinical trials involving patients with glioma, Kaposi's sarcoma (KS), prostate cancer, breast cancer, and multiple myeloma. "We had speculated that there are five metabolites and, after



Fran Poliner

Giovanna Tosato (left) and Hynda Kleinman confer after session

proaches (radiation, hormonal, surgery, chemotherapy). That would be the optimal approach." He noted that there are two trials (on campus) testing such combinations in prostate cancer patients: one of docetaxel (Taxotere) and thalidomide and the other of leuprolide acetate (Leupron) and thalidomide.

### The Genesis of Angiogenesis?

Her laboratory and animal work have suggested to Hynda Kleinman that Thymosin beta-4 (T $\beta$ 4) may be "the angiogenic molecule in metastasis." Prospecting for the genes through which the angiogenic properties of endothelial basement membrane cytokines are activated, Kleinman has found ample evidence for a pivotal role for T $\beta$ 4.

In studies of endothelial cells undergoing early steps in angiogenesis, Kleinman, who is chief of the NIDCR Cell Biology Section, found one gene—T $\beta$ 4—that was upregulated and later shown by her group to be angiogenic. This molecule, she noted, was recently found by others to be one of three genes consistently upregulated in metastatic tumors.

T $\beta$ 4, Kleinman summed up, is "present in wound fluid and metastatic tumor cells. It promotes the migration of coronary artery endothelial cells. In the rat model, it works to re-epithelialize the corneal eye surface. Tested topically and intraperitoneally in rats, it decreases wound width amazingly."

She sees therapeutic potential for T $\beta$ 4 in treating wounds in elderly, diabetic, and other patients with impaired wound healing and (in collaboration with investigators from NEI, the Uniformed Services University of the Health Sciences in Bethesda, Md., and George Washington University in Washington, D.C.) is on the threshold of a clinical trial to test that expectation. ■

several years, we have finally synthesized one—MW3, a 5'OH metabolite of thalidomide—that does inhibit angiogenesis in some of our model systems," he said.

Clinical results among KS, multiple myeloma, and prostate cancer patients have been encouraging, Figg said, noting that declines in prostate-specific antigen in the prostate cancer cohort, all of whom had failed hormone ablation and nearly all of whom had bone metastases, are correlating with improvements seen on PET scan.

However, he added, "don't oversell this approach. It looks like angiogenesis needs to be combined with other antitumor ap-

### Turning to Gene Therapy

Steve Libutti, a clinical investigator in NCI's Surgery Branch and chair of a clinical trial of thalidomide in patients with recurrent colorectal cancer, focused on the harnessing of endogenous antiangiogenic agents as an anticancer strategy.

He noted that scientists have been unearthing endogenous inhibitors of the endogenous promoters of angiogenesis (such as vascular endothelial and fibroblast growth factors) at an "exponential rate."

The reasons for this anti-angiogenesis drive, he said, are clear: Blood vessels are similar across all tumor histologies, resistance does not appear to be an issue



Fran Poliner

William Figg

because endothelial cells have low mutagenic potential, toxicity appears to be minimal, and there may be synergy with conventional cancer therapy.

At this point, he added, the main disadvantage is the need for chronic administration, because blood vessels will resume growing upon discontinuation of the angiogenesis inhibitor. His solution is "allowing the host to become a factory" of the chosen therapeutic agent—endostatin, in the studies he's involved in—through gene therapy.

"We have not discovered the perfect vector system yet," he said, summarizing the relative merits of tumor-directed



Fran Pollner

Steve Libutti

vs. systemic delivery. The latter might be selected for patients with micrometastases. In mouse studies using recombinant adenovirus and murine endostatin, delivered intravenously into the liver, sustained circulating endostatin yielded 40

percent inhibition of tumor growth in injected human melanoma cells; adenovirally delivered pigment epithelial derived factor also inhibited mesothelioma growth in another animal study. Neither intranasal nor intraperitoneal routes of delivery were successful.

Tumor-targeted delivery, he continued, would be suited to the large-tumor setting. For this purpose, he and his team have explored vaccinia virus, which selectively targets tumor tissue. In a mouse model, pretreatment with recombinant vaccinia-EMAPII, a tumor-derived cytokine, yielded diminished tumor size after TNF injection.

The team is now testing an attenuated salmonella vector, an as yet "untapped" vector source whose sensitivity to antibiotics, Libutti observed, would represent a "safety margin" in forthcoming clinical trials should the vector need to be eliminated for any reason.

The team is embarking on a Phase I clinical trial involving patients with metastatic melanoma that will test the attenuated vector alone. If it proves to target to human tumors, "we'll send in a gene," Libutti said. ■

## RECENTLY TENURED

**Joan Bailey-Wilson** earned her Ph.D. in medical genetics with a minor in biomathematics from Indiana University in 1981. She then pursued postdoctoral training in statistical genetics with Robert C. Elston at the Louisiana State University Medical Center in New Orleans, where she joined the faculty in 1982 and rose to the rank of full professor in the Department of Biometry and Genetics before joining NIH in 1995. A diplomate of the American Board of Medical Genetics and founding fellow of the American College of Medical Genetics, she is currently a senior investigator and head of the Statistical Genetics Section in the NHGRI Inherited Disease Research Branch. She is also an adjunct full professor in the Department of Epidemiology at the Johns Hopkins University School of Public Health in Baltimore.

Over the course of my career, I have had a strong interest in the statistical genetic study of complex diseases, with an emphasis on human cancers—particularly lung, breast, colon, and prostate cancers. I have published segregation analyses predicting major susceptibility loci for breast cancer, HNPCC colon cancer, and lung cancer, as well as linkage studies implicating such loci for prostate and breast cancer.

My lung cancer analysis gave the first evidence for a Mendelian locus that may act in conjunction with smoking to influence lung cancer risk in some families. This was a major paradigm shift and stimulated other scientists in the field to do other segregation analyses that have shown similar results.

I am currently co-PI of a large collaborative linkage study of lung cancer and am PI of NIH studies of lung cancer and of oral clefts, as well as co-investigator on linkage studies of several other complex diseases, including prostate cancer, breast cancer, colon cancer, melanoma, myopia, inflammatory bowel disease, and familial intracranial aneurysm.

While applied research is of particular interest to me, I am also involved



Joan Bailey-Wilson

in various theoretical projects. The effect of misspecification of trait and marker models on the power and type I error rate of linkage statistics has been one of my strong theoretical interests for many years. My students and I have found that Haseman-Elston sib-pair linkage analysis is robust to misspecification of

marker allele frequencies but that parametric lod-score linkage methods are not robust in the same situation—an important theoretical discovery.

We are currently pursuing the causes of this lack of robustness of parametric lod-score methods and devising methodological strategies to overcome it. We are also trying to determine which other popular statistical genetic analysis methods are not robust to misspecification of marker allele frequencies.

My interest in lung cancer genetics led me to a study of the effects of environmental covariates on the power of linkage. I have shown that existing methods of incorporating measured environmental exposures into Haseman-Elston sib-pair linkage are not powerful and have developed an analytic strategy that greatly improves power to detect a major locus when environmental exposures are important in the etiology of a complex disease.

This methodology is critical for adequate power to detect genes in complex traits, such as lung cancer, for which environmental exposures (such as cigarette smoke) are the most important causative factors.

Collaborative work on the search for susceptibility genes for prostate cancer, breast cancer, and melanoma have sparked an interest in extending current methods of family-based association analysis. These applied studies have produced evidence for linkage in various regions, and some of the data are particularly appropriate for linkage disequilibrium studies.

These studies are aimed at narrowing candidate regions for the location

*continued on page 12*

### For More Angiogenesis Info

■ NICHD investigator Brant Weinstein has produced a videotape of angiogenesis in the zebrafish that tracks two signaling pathways and compresses 10 hours of blood vessel formation into a few minutes; he also has established a website on the vascular anatomy of the zebrafish:

<<http://eclipse.nichd.nih.gov/nichd/lmg/redirect.html>>.

■ An NIH interest group on Tumor Angiogenesis and Invasion can be found at

<<http://www.nih.gov/sigs/sigs.html>> and click onto group name.

■ For a list of angiogenesis inhibitors in clinical trials in the NCI trials database, see

<<http://cancertrials.nci.nih.gov/news/angio/angiomore.html>>.

of susceptibility genes. I have recently extended the Sibling-TDT methodology to allow analysis of an X-linked trait and have published these results in a joint paper with Gloria Ho, who extended the TDT in a similar manner.

I am also working with James Malley of NIH's CIT to develop new likelihood-based bootstrap methods of association analysis that not only test for both linkage and association but are as powerful as or more powerful than the TDT and give estimates of both the association parameter and the linkage recombination fraction.

Traditionally, estimates of the recombination fraction have not been available from association data (either case-control or family-based). Thus, this new method represents a major advance in the field of family-based linkage disequilibrium mapping.

In the future, I intend to continue my focus on the development and testing of methods for the statistical genetic analysis of complex diseases. I also intend to continue my search for genetic loci that increase susceptibility to such complex disorders.

**Richard Koup** received his M.D. from The Johns Hopkins University School of Medicine in Baltimore in 1982. He was a staff investigator at the Aaron Diamond AIDS Research Center in New York City before becoming chief of infectious diseases and professor of medicine and microbiology at the University of Texas Southwestern Medical Center, Dallas. He will be joining the Vaccine Research Center this summer as the director of the Human Immunology Program and chief of the Laboratory of Immunology.

I have been actively involved in HIV vaccine-related research for many years. My studies have focused on the role of HIV-specific cellular immunity in controlling HIV infection. I described the temporal association between the emergence of the cytotoxic T-lymphocyte (CTL) response to HIV and the initial control of viral replication in human infection, and I am the co-discoverer of a natural genetic mutation in the CCR5 co-receptor for HIV that provides significant protection against infection.

Most recently, my colleagues and I

have shown that the adult thymus continues to produce naïve T cells and contributes to immune reconstitution in patients with treated HIV infection or patients who have undergone hematopoietic stem cell transplantation.

My lab's long-term goal is to determine the influence of the cellular immune response on containment of HIV replication. The ultimate loss in immune control of HIV contributes greatly to disease progression, and a clear delineation of the processes leading to this loss of control will be crucial to the development of a protective, or at least effective, immune response to a vaccine.

The most practical way to achieve this in humans is to characterize—precisely and quantitatively—the correlations between fundamental aspects of the HIV-specific T-cell response and clinical and virologic parameters of HIV disease. This involves the analysis of the frequency, function, and specificity of HIV-specific T cells in response to HIV peptide antigens. We accomplish this primarily through intracellular cytokine analysis and a novel technique we developed that combines in vitro antigen stimulation and real-time quantitative clonotypic PCR.

HIV may evade cellular immune control through sequence variation within recognized epitopes. In addition, HIV may evade immune control by destroying CD4<sup>+</sup> T cells needed to maintain the CTL response. To study this possibility, we are using newer methods that enable us to quantify the number, phenotype, and function of

HIV-specific CD4<sup>+</sup> and CD8<sup>+</sup> T cells. These methods include a combination of intracellular cytokine staining, cytokine ELISA or Elispot, tetramer staining, and functional assays of cytotoxicity and T-cell division. With these tools, we can extensively characterize the T-cell response to HIV in patients in various stages of infection and thereby delineate which defects in immune response are leading to loss of control of HIV replication.

The classical role of a vaccine is to stimulate immunity that will protect against subsequent infection. In the case of HIV, there is another potential role

for a vaccine: Because progressing HIV infection entails loss of immune control of the virus, a vaccine could be used to boost waning immunity and restore control of virus replication.

We plan to test the ability of therapeutic vaccination to alter virologic control in HIV-infected subjects by developing vaccines that boost specific cellular responses that are defective in HIV-infected subjects. To enable this and other vaccine research, we are also working to adapt assays of cellular immune function for high throughput screening and monitoring of clinical vaccine trials. I look forward to bringing this research to NIH this summer and sharing ideas and methods with investigators throughout the intramural research program.

**Roland Martin** received his M.D. in 1982 from the University of Würzburg Medical School in Würzburg, Germany, where he then did postdoctoral work in the Department of Virology and Immunobiology and completed a residency in neurology. From 1989 to 1991, he was a postdoctoral fellow in the Neuroimmunology Branch, NINDS, and from 1991 to 1994 he worked as an assistant professor of neurology and clinical attending in the Department of Neurology at the University of Tübingen, Germany. From 1994 until 1996, as the recipient of a Heisenberg research professorship of the German Research Society, he supervised laboratories at the Neuroimmunology Branch, NINDS, and in Tübingen. He then spent a year in the neurology residency program at the University of Maryland at Baltimore and in 1998 joined the Neuroimmunology Branch, NINDS, as a tenure-track investigator. He is now acting chief of the Cellular Immunology Section.

From my time at medical school and later during fellowships in virology, immunology, and neuroimmunology, I have always had a strong interest in infectious and inflammatory diseases

of the nervous system and in clinical neurology. Multiple sclerosis (MS) appeared the best field to apply and expand these interests. During the past 15 years, my work has focused on cellular immune function and antigen rec-



Fran Pollner

Richard Koup



Fran Pollner

Roland Martin

ognition in MS and also on nervous system manifestations of Lyme disease.

In Henry McFarland's laboratory, we were among the first to show that T-cell reactivity against myelin components is observed not only in MS patients but essentially in every individual. This puzzling observation indicated that thymic negative selection is incomplete and that autoimmune T-cell responses may develop in every individual. Because this usually does not occur, much less lead to disease, scientists now assume that the appropriate immunogenetic background and an exogenous trigger, such as a viral infection, are probably required to induce pathogenic autoimmunity.

Using novel peptide chemistry approaches, we were able to demonstrate that T cells are capable of responding to a broad range of antigens—in some cases, with very high sensitivity. Combinatorial peptide libraries in the positional scanning format allowed us to identify systematically molecular mimics for autoreactive T-cell clones or for T cells from chronic infectious disorders such as Lyme disease.

An additional focus of our laboratory has been directed toward the development of novel immunomodulatory therapies for MS. A recent trial with an altered peptide ligand derived from an immunodominant peptide was disappointing from the therapeutic point of view because we were not able to show clinical benefit. However, we learned an important lesson from this trial. Some patients suffered exacerbations under peptide therapy, indicating that this myelin peptide is indeed an immune target in MS and that pathogenetic principles that have been deduced from animal experiments and human *in vitro* studies are valid.

Currently, the Cellular Immunology Section focuses on three major areas: the continuation of our studies of T-cell antigen recognition in autoimmune and inflammatory diseases, identification of disease-related genes and biomarkers in MS, and the development of novel treatment strategies for MS.

In our new approaches to MS treatment, we plan to combine immunomodulatory approaches with repair strategies such as stem-cell transfer. NIH offers an ideal collaborative environment to study basic mechanisms in human diseases in collaboration with other groups, translate this knowledge into new treatments,

and eventually apply them in novel experimental trials.

**Jonathan Vogel** received his M.D. from Rush Medical College, Chicago, in 1981. He completed an internal medicine residency at Barnes Hospital, Washington University Medical Center, St. Louis, and a fellowship at the NCI Laboratory of Molecular Virology. He held a faculty position at the Holland Laboratories, American Red Cross, Rockville, Md., before joining the NCI Dermatology Branch in 1992, where he is now a senior investigator.

The long-term goal of my laboratory is to understand how to introduce and successfully express genes in the skin in a manner that is therapeutically useful for clinical applications. For most purposes, this will require that the desired gene be expressed long enough in a significant percentage of keratinocytes.

Achieving persistent expression in a renewable tissue such as skin remains a challenge and will require stable gene insertion into keratinocyte stem cells. Our current laboratory efforts are focused on identifying and enriching keratinocyte stem cells, efficiently introducing genes into stem cells by genetic manipulations, and topically selecting *in vivo* for keratinocytes that express the transgene after they have been grafted onto a recipient.

In my earlier work at NIH, we developed a novel approach for directly introducing and transiently expressing genes in epidermis simply by injecting naked plasmid DNA into the dermis. In a series of studies, we characterized the uptake and expression of plasmid DNA in human, pig, and mouse skin. These studies showed that biological response modifiers, such as cytokine genes, could be expressed in the epidermis and achieve an expected biological effect, such as the recruitment of neutrophils.

Although skin gene expression with this direct approach was transient, we found it to be very effective for DNA vaccination. We next demonstrated that DNA vaccination with plasmids expressing *Leishmania* proteins can provide protection against *Leishmania* infection in susceptible mice due, in part, to the presence of immunostimulatory DNA

sequences (nonmethylated CpG dinucleotides flanked by two 5' purine nucleosides and two 3' pyridine nucleosides) on the plasmid DNA.

Furthermore, oligodeoxynucleotides containing these immunostimulatory sequences were found to be effective adjuvants when combined with *Leishmania* protein antigens and were able to protect susceptible mice from infection. The adjuvant effect of these CpG-containing oligodeoxynucleotides could be explained by their activation of the dendritic antigen-presenting cells in the skin (such as Langerhans cells).

In the laboratory, skin organ cultures are an important tool for investigation. To help us understand the regulation of epidermal differentiation in these cultures, we have investigated how an epidermal-specific transcription factor, the POU family transcription factor Skn-1, regulates keratinocyte differentiation. In these studies, we characterized the transactivating and inhibitory domains of Skn-1 by mutational analysis. We demonstrated that Skn-1 enhances keratinocyte differentiation, the expression of differentiation-specific markers, and keratinocyte proliferation.

Although we have successfully introduced and transiently expressed genes in the skin in the studies described above, achieving long-term expression of desired genes in a significant percentage of keratinocytes has proved to be very difficult. Currently, it is not possible to introduce desired genes efficiently into keratinocyte stem cells. To address this issue, we are pursuing three complementary approaches.

First, we have developed a model using topical treatments to select for keratinocyte stem cells that contain and express a selectable marker gene linked to a gene of interest. Second, we will evaluate whether different viral vectors, such as lentiviral vectors, can introduce a desired gene into keratinocyte stem cells more efficiently than the retroviral vectors traditionally used. Finally—and perhaps most importantly—we are trying to identify unique keratinocyte stem cell surface markers (or patterns of gene expression) so that the keratinocyte stem cells can be purified and enriched for efficient genetic manipulation. ■



Fran Pollner

Jonathan Vogel

## SPEAKING OF SCIENCE . . . .

The NIH Speakers Bureau started 10 summers ago with a list of six names—six friends of then—Office of Education director Michael Fordis, who'd been getting calls from local groups asking for speakers. "He knew these friends of his were good speakers, so he put them on a list and gave the list to me and asked me to organize a speakers bureau," Gloria Seelman, now with the Office of Science Education (OSE), recalls.

Today the Speakers Bureau is under OSE jurisdiction and is administered by Anne Baur, who calls it "one of NIH's best kept secrets"—but a secret that has a website

<<http://science-education.nih.gov/spkbureau.nsf/>> that has had its share of hits every day since its launch in 1998. Those who click will find a directory of speakers (with their profiles, their fields of expertise, and questions they are eager to address) and an extensive list of specific medical and ethical topics that Bureau troops are ready, willing, and able to talk about.

At the moment, Baur says, there are 61 speakers in the directory, but she hopes to have "100 by the start of the school year [2001-2002]" and to expand marketing efforts beyond the current "word-of-mouth" that generates about 180 calls for speakers each academic year.

The speaking venues are diverse, ranging from elementary schools to retirement communities. And someone who signs up as an expert in a particular medical field can find herself or himself adapting the information for an audience of curious children, concerned consumers, or prospective scientists, to name a few possibilities.

Everybody associated with the project benefits, Baur says. The Speakers Bureau supports the OSE and NIH mission of disseminating medical information and contributing to the public's understanding of medical research; each speaker gets the opportunity to develop a variety of effective verbal communication skills while, often, expounding on his or her own scientific achievements; the audience gets to hear state-of-the-art scientific achievements



Anne Baur

"straight from the horse's mouth"; and the program sponsor, the Office of Research on Women's Health, has another outlet to showcase accomplished women and minorities in science.

NCI's Kathleen Higinbotham is one such volunteer who last year spoke of her experiences as both a breast cancer researcher and breast cancer survivor at a women's health symposium organized by Sara Grove, an associate professor of political science at Shippensburg (Pa.) University, and her students. A student who had undergone chemotherapy for Hodgkin's disease and awaited radiation therapy found Higinbotham's talk "encouraging and amazing," Grove says, adding that she found the Speakers Bureau website "very user-friendly and im-

pressive." She plans to use the resource again.

Women's health, Baur says, is one of the topics that commands the greatest interest and, within that realm, osteoporosis and breast cancer are the most frequently specified. She would especially like to expand the bureau's roster of speakers in this area.

To become a full-fledged NIH Speakers Bureau speaker, one need only be an interested NIH scientist, administrator, or support staff—or, actually, an employee of any federal agency that promotes science and health research. Participation in the program is considered official duty for which a speaker may not accept compensation other than to cover travel expenses.

Once someone signs up for service, he or she can expect to be called upon two or three times a year. "If anyone is so popular that their requests are interfering with their job performance, I can take them [off the list] and give them a rest," says Baur, adding, however, that

### Never Too Old

A "commitment to help shape public understanding of genetic research" brought Barbara Biesecker, head of NHGRI Medical Genetics Branch and co-director of the collaborative Johns Hopkins University-NHGRI Genetic Counseling Graduate Program, to the Speakers Bureau. She particularly enjoys speaking with senior groups. "Their collective life experiences lead them to ask informed questions and appreciate the subtleties of ethical dilemmas," she says, adding a more personal reason for enjoying her interchanges with older people: While handshakes, smiles, and verbal "thank yous" are usually forthcoming wherever she speaks, in the company of her elders, she has also been hugged and treated like a daughter. ■



Barbara Biesecker

### . . . Or Too Young

Alfred Johnson, director of the NIH Undergraduate Scholarship Program and an investigator in the NCI Laboratory of Molecular Biology, was already speaking before he joined the group. But, he says, it's "nice to have a formal process in place to assist the effort." He speaks to students in kindergarten up through high school and to adults about cancer-related topics and about careers in science. He recalls an especially poignant moment when a third grader asked why people die, a question that eluded a satisfactory scientific answer but somehow evolved into an exchange that called forth genuine group laughter.

Having students tell you that you have "sparked their joy in science is overwhelming," he says. ■



Alfred Johnson

by Cynthia Delgado, OSE

the speakers have complete control over which requests they accept or decline, as well as how long they will participate in the program. (The website is set up to enable direct e-mail communication between a requestor and a selected speaker.)

To volunteer as a speaker, request a speaker, or learn about other OSE programs and resources visit the website:

<<http://science-education.nih.gov>>.

## Give and Take

Maria Giovanni, assistant director for microbial genomics, NIAID, and organizer of the Bethesda Elementary after-school science club, invited NCI's Marian McKee, a staff scientist in the

Biotherapy Section of the Laboratory of Molecular Biology, to the club. "We were looking for hands-on activities, not another lecture," says



Marian McKee

Giovanni. What they got was a creative painting project that showed the way microorganisms are transmitted from one person to another. The best part, Giovanni recalls, was McKee's enthusiasm, a characteristic to which the kids really respond. "It was a very positive experience; [the kids] are a critical group, and they really liked it."

For McKee, the best part of talking to elementary school students is their enthusiasm and their curiosity. She is "struck," she says, by "how thoughtful many of the questions are, even from the younger (K-2) students." She's remained on the speaker list, she says, because she enjoys "demystifying the world of the white-coated lab worker" for the children. There's also the fact that she invariably returns to the lab "in a more positive frame of mind." ■

## Phases of Life

Upcoming "Faces and Phases of Life" seminars, presented by the Work and Family Life Center (WFLC) and the Employee Assistance Program, include:

**Thursday, March 29, 12:00-4:00, 31/6C10.** "Estate Planning."

**Wednesday, April 4, 12:00-1:30, 31/6C6.** "Setting Limits and Delegating."

**Tuesday, April 10, 12:00-2:00, 31/6C6.** Taking Charge of Your Career.

**Thursday, April 19, 12:00-1:30, 31/6C6.** "Walking the Gauntlet: Coping with Life."

**Tuesday, April 24, 12:00-1:30, 1/151** (note this change from the original location of 31/6C6). "Transitions, Part 2. Keeping the Faith—Midlife Transition Issues and Beyond."

**Wednesday, May 2, 12:00-1:30, 31/6C6.** "Paying for Care for Older Relatives. Part I: Original Medicare."

**Thursday, May 3, 12:30-2:00, NSC Conference Room D, 6001 Executive Boulevard** (NOTE: this seminar will not be available via videocast). "The Basics of Balancing Work and Family."

**Tuesday, May 8, 12:00-1:30, 31/6C10.** "Paying for Care for Older Relatives. Part II: Additional Options."

**Wednesday, May 16, 12:00-2:00, 31/6C6.** "Preparing KSA Statements for a Federal Career."

**Wednesday, May 23, 12:00-1:30, 1/Wilson Hall.** "Stop Putting it Off!"

**Wednesday, May 30, 11:30-1:30, 31/6C6.** "Bringing Balance to Our Fragmented Lives."

All seminars are free. Any changes will be posted at the WFLC website and sent to individuals on the e-mail list. Call WFLC at 301-435-1619 to register, be placed on an e-mail list, or arrange videoconferencing to your location. Most seminars may be watched at the NIH video-casting website, either in real-time or from the archive

<<http://videocast.nih.gov>>.

The videocassette may also be rented from the WFLC resource collection—it will be available about two weeks after the workshop.

Sign language interpretation is provided. For other reasonable accommodations, call the WFLC 48 hours before the seminar (301-435-1619, TTY/TDD: 301-480-0690). ■

## Who's the FAREest?

Fellows are invited to submit an abstract of their current research to the eighth annual FARE (Fellows Award for Research Excellence) competition. FARE recognizes outstanding intramural scientific research and is open to postdoctoral IRTAs, visiting fellows, and other fellows with less than five years total postdoctoral experience in the NIH IRP. Pre-IRTAs performing their dissertation research at NIH are also eligible to compete. Visiting fellows and scientists must not have been tenured at their home institute. Questions about eligibility should be addressed to your institute's scientific director.

Abstracts are evaluated anonymously on scientific merit, originality, experimental design, and overall quality and presentation. Twenty-five percent of the fellows who apply will win an award. Winners will be announced by September 2001 and receive a \$1,000 travel award to use for attending and presenting their work at a scientific meeting between October 1, 2001, and September 30, 2002. Fellows are asked to submit their application, including abstract, electronically from **May 1 to May 31, 2001 (5:00 p.m., EST)**, via the NIH Fellows Committee website:

<<http://felcom.nih.gov/FARE>>.

Those who cannot access the electronic application in their laboratory can find additional computers in Bldg. 10 at the NIH Library, the CC Information Technology Center (Room 1C282), and the CyberCafe/Graduate Student Lounge; and in Bldg. 31 at the User Resource Center (Room B2B47).

## Caregiver Fair

The HHS Administration on Aging is holding an "Older Americans Caregiver Fair" **Tuesday, May 1, from 10:00 a.m. to 2:00 p.m.** at the Humphrey Building Great Hall (200 Independence Avenue, S.W.) in Washington. Find out about home-delivered meals, transportation, Medicare/Medicaid, daycare and respite care, long-distance care-giving, and more. For more fair information, contact: Irma Tetzloff at 202-619-3268; or Tom Northam at 202-401-9647; or e-mail:

<[Irma.Tetzloff@aoa.gov](mailto:Irma.Tetzloff@aoa.gov)>:

<[Thomas.Northam@aoa.gov](mailto:Thomas.Northam@aoa.gov)>.

## CALL FOR CATALYTIC REACTIONS

In this issue, we are asking for your reactions in four areas: scientific misconduct, attracting postdocs, proteomics progress, and the NIH Speakers Bureau.

**Send your responses on these topics or your comments on other intramural research concerns to us via e-mail:**

**<catalyst@nih.gov>;  
fax:402-4303; or mail:  
Building 2, Room 2W23.**

### *In Future Issues...*

- Proteomics—Part 2: Drug Monitoring
- New NCI Fellowship Office
- Gene Silencing

1) Is the federal government headed in the right direction in its approach to defining and managing allegations of scientific misconduct?

2) What steps would you advise investigators to take to attract the postdocs of their choice?

3) Tell us your experience with proteomics—are proteomics resources that are available now truly “hot methods” or not yet ready for prime time?

4) Would you sign on as an NIH Speakers Bureau volunteer? Why or why not?

*The NIH Catalyst* is published bi-monthly for and by the intramural scientists at NIH. Address correspondence to Building 2, Room 2W23, NIH, Bethesda, MD 20892. Ph: (301) 402-1449; fax: (301) 402-4303; e-mail: <catalyst@nih.gov>

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